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OBSERVATIONS ON THE COMMON INTESTINAL PROTOZOA OF MAN: THEIR DIAGNOSIS AND PATHOGENICITY.¹

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(With Plates.)

At the present time, while there is a great influx of individuals into this country who have been residing for a longer or shorter period in tropical or sub-tropical countries, certain types of infection, which usually only come to the notice of those interested especially in tropical medicine, are likely to be encountered by many who have not had any previous opportunity of studying the organisms present. Having had during a period of ten years as protozoologist to the London School of Tropical Medicine abundant opportunity of studying the protozoal infections of man both in this country at the Albert Dock Hospital attached to the Tropical School and in many parts of the world abroad, I have thought it useful to give a short account of one group of protozoal organisms which infect human beings, especially in warm climates. I refer to those protozoa which inhabit the digestive tract, some of which are definitely pathogenic and invade the tissues, others which are less certainly injurious, and, finally, some against which it is difficult to produce any evidence of harmful action whatever.

The protozoa which are to be found in the intestine belong to three² of the great subdivisions into which these organisms are grouped. Belonging to the Rhizopoda we have the definitely pathogenic *Entamoeba histolytica*, the quite harmless *E. coli*, and a small amoeboid organism which on account of its resemblance to the free-living water amoeba, *Amoeba limax*, is often considered to be actually this amoeba or one nearly related to it which has taken up a temporary existence in the human intestine. All these inhabit the large intestine and caecum.

To the group of the Mastigophora belong three flagellates which are commonly to be found in the human intestine—*Trichomonas*

¹ Reprinted from the *Lancet*, November 27, 1915, with a new Appendix.

² Quite recently a Coccidium belonging to the group Sporozoa has been found in the cases from Gallipoli (see *Coccidiosis* at end of paper), so that members of all four subdivisions of the Protozoa are to be found in the human intestine.

intestinalis, *Tetramitus mesnili*, and *Lambliia intestinalis*. Of these *L. intestinalis*, inhabiting the upper part of the small intestine, has the greatest claim to being pathogenic; *T. mesnili* has been supposed to give rise to intestinal irritation followed by diarrhœa, while *T. intestinalis* is generally regarded as quite harmless, though Escomel in South America claims to have cultivated this organism and to have produced diarrhœa in dogs by injection of these cultures. The two latter flagellates inhabit chiefly the large intestine and cæcum, and are to be found in greatest number when the intestine is deranged for some reason or another, for the change in the intestinal contents resulting from such diseases as typhoid, dysentery, etc., seems to induce a very active multiplication of these organisms. There are other flagellates which are much less frequently met with. These are species of *Bodo*, *Cercomonas*, and *Prowazekia*, simple organisms, each with two flagella, which occur especially in diarrhœic conditions and which are possibly only free-living flagellates which have found a suitable medium of development in the altered condition of the gut. Several members of the group Ciliata have been described, but only one of these has any claim to being a common parasite. This one is *Balantidium coli*, a large organism which lives in the large intestine. Pigs are generally infected with this ciliate, which, escaping from them in the encysted condition, gains entrance to the human intestine, and by actual invasion of the wall of the large intestine produces the serious condition known as balantidial dysentery.

In this paper I wish to give a short account of these various protozoa, more especially from the point of view of diagnosis and the methods I have found useful for their identification. That such identification is important goes without saying, for on it may depend the actual treatment of any individual case.

Some years ago I read a paper before the British Medical Association on the subject of the intestinal entamœbæ of man and their differentiation. I showed there that the pathogenic *E. histolytica* was most readily distinguished from the harmless *E. coli* by the examination of the encysted forms—forms which have protected themselves by the secretion of resistant transparent capsules which will enable them to withstand exposure after their escape from the human intestine and until they can succeed in gaining entrance to another individual. Now with all the intestinal protozoa of man, with the possible exception of *T. intestinalis*, infection spreads from one individual to another by means of encysted forms which,

as far as we know, undergo no development outside the body, but simply lie about in water till they are ingested by another individual. If by any chance an unencysted protozoon should escape from the intestine, and this happens very commonly when infected individuals have attacks of diarrhoea which flush out the intestine, such forms invariably die quickly, and it is difficult to see how they can be responsible for the spread of infection. In the case of *T. intestinalis*, however, encysted forms have not been definitely described, and it is perhaps owing to this that *T. intestinalis* survives in fæces it may be a week or more after escape from the body in the unencysted condition. With this possible exception, in the normal course of events it is only the encysted forms of these protozoa which escape in the fæces and are destined for future development in another human host. Manifestly, then, it is important to be able to recognize not only the free-living forms of the intestinal protozoa as they move about in the intestine, but also the encysted forms which, corresponding to the eggs of worms, may in the same way be the only indications that there is an infection of the intestine with any particular organism. The finding of the eggs of *Ankylostoma duodenale* or of *Ascaris lumbricoides* in the fæces of an individual shows that the intestine is harbouring these worms, and similarly the discovery in the fæces of the encysted forms of *E. histolytica* or *L. intestinalis* shows that an infection with these protozoa exists and, in the case of the former at any rate, calls for energetic treatment, for such an infection may be the cause of the spread of amoebic dysentery to other individuals, or may be followed by a fatal attack of dysentery in the person himself.

Naturally enough, the intestinal protozoa are usually encountered when there is some definite intestinal derangement, for it is only in such conditions that the fæces are submitted to examination. The finding of these comparatively large and active organisms, sometimes in enormous numbers, is likely to lead to the conclusion that they must be the determining factor of any intestinal symptoms which may be present. The findings can only be controlled by the examination of normal individuals. In tropical countries frequently over fifty per cent of the normal population harbour *E. coli*, and Schaudinn showed some years ago that in certain parts of Germany a still larger percentage were infected. Similarly, in tropical countries flagellate infections of the intestine are exceedingly common apart from symptoms, so that when they are met with in cases of diarrhoea one must very carefully exclude all other causes,

bacterial or irritant, before concluding that flagellates have produced the condition in question. In the case of *E. histolytica* and *B. coli* there are produced by these protozoa extensive lesions of the bowel, in the tissues of which they may be found. They wander far afield and produce lesions in the mesenteric glands, liver, and other organs, while, experimentally, animals may be infected and in them are produced lesions similar to those in man. In these cases it is impossible to doubt the pathogenicity of the protozoa. In the case of the flagellate infections, however, no definite lesions of the gut occur, and as no satisfactory post-mortem material can be obtained owing to the rapid degeneration of the protozoa after the death of the host it is very difficult to obtain any definite proof of pathogenicity. Experimental infections in animals have not given very satisfactory results, and no one has yet been able to obtain pure cultures of these flagellates. The subject is a difficult one and requires further investigation before we can definitely assert that the intestinal flagellates are pathogenic.

Another point which must be borne in mind is that the intestinal flagellates, in common with intestinal bacteria, sometimes invade the tissues shortly before or after death. This is due apparently to a diminished resistance on the part of the intestine, which permits the passage of organisms which normally only live in the lumen of the gut. Cases of this invasion are fairly common in animals. Gonder, for instance, found *Lamblia* in the blood-stream of a fowl, and quite recently Basile in Italy has noted a case of *Lamblia* of the liver of a rat, which was dotted over with white cysts containing a fluid in which the flagellates were living. Basile inoculated a rat with some of this fluid intraperitoneally and later discovered *Lamblia* in the liver and mesenteric glands. These are exceptional cases, but so long as they occur there is the possibility that the invading flagellates will give rise to symptoms of one kind or another.

I will now give a description of the various protozoa mentioned above, the method of their detection, and what is known of their habits and pathogenicity.

Entamæba coli (figs. 27 to 32).

This amoeba was first satisfactorily described by Casagrandi and Barbagallo, and it is perhaps the commonest protozoon of the human intestine. It seems to be a perfectly harmless organism, and in many parts of the world more than half the healthy population are infected. It inhabits the large intestine and cæcum, where

it crawls about by its amoeboid movements amongst the intestinal contents and more especially over the surface of the gut. It consists of a mass of cytoplasm which varies in diameter from ten to thirty microns, though larger or smaller forms may occur. It has a single large spherical nucleus consisting of a nuclear membrane on the surface of which are irregular masses of chromatin which appear as greenish refractile granules in the living condition. At the centre of the nucleus a granule, the karyosome, can be seen. The cytoplasm is often very much vacuolated, the vacuoles containing bacteria, yeasts, and even encysted and unencysted flagellates, which the amoeba has ingested. It is most vacuolated and granular towards the centre and becomes less so towards the periphery, but there is, as a rule, no sharp distinction between ectoplasm and endoplasm. The whole organism tends to have a greyish colour, is of liquid consistency and not highly refractile. When it moves it throws out pseudopodia, which are blunt structures, and in these again no clear line of demarcation can be made out between ectoplasm and endoplasm, unless at the very commencement of pseudopodium formation. The nucleus can usually be seen clearly in the living condition unless it is obscured by vacuoles and their contents, and it changes its position in the cytoplasm with the movements of the amoeba. The amoeba even when observed in the warm stage moves very slowly, so that occasionally the only sign of life is a gradual change in shape.

Reproduction is by simple division. The single nucleus divides, and this is followed by division of the cytoplasmic body. The two amoebæ thus formed increase in size and later divide again. In this way the infection of the large intestine is maintained. The size of the amoebæ depends largely on the rate of multiplication, for if the divisions succeed one another rapidly before there has been time for growth to the original size then the amoebæ tend to decrease in size, and conversely when division is slow they tend to be large.

As reproduction is proceeding in the large intestine certain individuals cease to multiply; they discharge all their food contents and, becoming spherical, secrete round themselves a protective covering or cyst. This is a transparent capsule very much like, but not so thick as, the shell of an ankylostome egg. When encystment has taken place a passive body is produced, which is carried down the intestine and eventually is discharged in the faeces. During the passage down the large intestine the single nucleus possessed by the encysting amoebæ divides into two, each of these divides

again to produce four, and the four again to produce eight (fig. 31). Cysts may be discharged in any of these stages of development, but most usually it is the fully developed cyst containing the single mass of cytoplasm in which are embedded eight nuclei which is met with in the fæces. The size of the cyst of *E. coli* varies between 15 and 20 microns, though slightly larger and very exceptionally smaller ones occur. After escape from the body they undergo no change till they enter a new host, probably in drinking water. Under the action of the digestive juices, according to the investigations of Casagrandi and Barbagallo, the contents of the cyst divide into eight small amœbæ, which escape into the intestine by rupture of the cyst. The fate of these small amœbæ has not been definitely traced, though it is suggested that they are in reality gametes or sexual forms which conjugate in pairs to form four amœbæ, which proceed to grow into the ordinary adult forms of *E. coli*. Schaudinn described a complicated sexual process (autogamy) as taking place in the cyst, but so far this has not received confirmation.

Infection of the intestine with *E. coli* is most readily recognized by the detection of the very characteristic encysted forms. The amœbæ themselves often occur in the fæces, but their differentiation, especially for the uninitiated, from *E. histolytica* may be a matter of great difficulty. The cysts, however, are easily recognized as clear refractile spherical bodies of very sharp outline which are readily seen with a $\frac{1}{6}$ or $\frac{1}{8}$ objective. In searching for them, and indeed for all intestinal Protozoa, it is essential to use only small quantities of material well diluted with water, or preferably saline and covered with a cover-glass. It is also necessary to be careful that the cover-glass is not floating about on the slide, for this will prevent satisfactory examination with the $\frac{1}{12}$ oil immersion. A rather dull light must be used, and I have found that the details can be more readily detected by reducing the light by lowering the condenser rather than by shutting out the light by means of the diaphragm. Under such conditions with $\frac{1}{6}$ objective it may be possible to detect within the cyst the characteristic eight nuclei. They appear as very faint greyish granular rings with a central dot or karyosome, and as the cyst is spherical they are not all in focus at the same time; to see them all one must focus up and down to bring different planes into view. The nuclei, however, are best seen by using the $\frac{1}{12}$ inch oil immersion lens and a No. 2 eyepiece. Here, again, it is generally necessary to reduce the light by lowering the condenser slightly. It is very important to see the nuclei properly, for on them depends

the absolute diagnosis of the cyst. They do not stand out in very great contrast to the cytoplasm within the cyst, but when once seen they are found to be quite sharply defined structures. They may be much more easily seen in one cyst than in another. The nuclei may be more clearly seen after the addition of a little iodine solution, as explained at the end of the paper. It is also important to determine the size of the cyst.

Occasionally abnormal forms occur. The commonest abnormality is the presence in the cytoplasm within the cyst of a large vacuole, which has the effect of pushing the nucleus to the side (fig. 32). It apparently hinders nuclear division to such an extent that these cysts frequently contain only two and rarely four nuclei. Another irregularity which I have very rarely seen are cysts in which there are sixteen instead of eight nuclei, while occasionally one meets with chromidial bodies like those so commonly seen in cysts of *E. histolytica*.

An individual infected with *E. coli* may retain the infection for years, as I have observed in some cases, and this in spite of treatment with intestinal disinfectants or treatment with emetine, which has such a marked effect in destroying the pathogenic *E. histolytica*. It is, perhaps, worthy of note that amœbæ closely resembling, if not identical with, *E. coli* have been found in rats, mice, monkeys, and other animals.

Entamœba histolytica (figs. 33-40).

Of all intestinal protozoa of man this one is the most dangerous in being the cause of amœbic dysentery and liver abscess. The dangers, however, are now largely reduced owing to the reintroduction by Rogers of emetine in the treatment of these diseases.¹ This drug appears to be a specific and has a most remarkable action upon *E. histolytica*, which disappears rapidly from the intestine when it is administered. This organism is undoubtedly the one studied and described by Lösch in Petrograd in 1873-4 under the name of *Amœba coli*, a name which later was employed for the non-pathogenic species.

E. histolytica differs little in size from *E. coli*, and, like it, is primarily an inhabitant of the large intestine. It has, however, the power of invading the tissues and multiplying in distant organs like the liver if it is carried there in the blood-stream. There are two distinct types of this amœba—one of the tissue-invading form

¹ *Lancet*, October 19, 1912, p. 1062.

known as the *tetragena* form, and the other a smaller form known as the *minuta* form, which lives, like *E. coli*, on the surface of the mucous membrane. The former is the one which is found in acute amœbic dysentery, when the stool may consist of little more than blood and slime. As the acute symptoms abate and the stools return to the normal the tissue-invading forms become replaced largely by smaller amœbæ, which appear to maintain the infection of the gut while tissue-invasion is in abeyance. They can often be found in numbers in stools which are quite or almost normal, though individuals thus infected are liable to repeated mild attacks of diarrhœa in which small quantities of blood and mucus are found, and they may at any time relapse into the acute dysenteric condition when the large tissue-invading forms reappear in the fæces. During the height of a dysenteric attack the only forms to be found in the fæces are the large tissue-invading forms about 15 to 30 microns in diameter (figs. 33 and 38). They are much more refractile and greenish-looking than *E. coli*; the spherical nucleus is smaller and has upon its membrane much finer granules of chromatin than that of *E. coli*, and on this account the nucleus is more difficult to detect in the living amœba. There is, further, a very clear distinction between ectoplasm and endoplasm, and when pseudopodia are formed they are found to consist entirely of highly refractile ectoplasm. The endoplasm may be vacuolated, but contains red blood corpuscles and leucocytes rather than bacteria, yeasts, and other foreign bodies. The presence of red blood corpuscles is a great aid to diagnosis, for *E. coli* does not ingest them. The movements of this amœba resemble, but are much more active than, those of *E. coli*. The tissue-invading forms multiply by simple division but do not encyst, in consequence of which encysted forms of this amœba are not found during a dysenteric attack. It might be difficult, therefore, to distinguish this amœba from *E. coli*, but for all practical purposes, if there are definite symptoms of dysentery with blood and mucus in the stool and large active amœbæ occur especially in the mucus, and some of them are found to contain red-blood corpuscles, it can be assumed that the case is one of amœbic dysentery due to *E. histolytica*. *E. coli* might conceivably occur under such conditions, but experience has shown me that this is practically never the case.

The greatest difficulty in diagnosis occurs when the acute symptoms have abated and the stools have almost returned to the normal. As already explained, under these conditions the large forms of the amœba are becoming replaced by smaller ones, which

vary in size from 10 to 20 microns (figs. 39 and 40). Unfortunately they often resemble *E. coli* closely, so that a diagnosis may be impossible if one had only the amœbæ to go by. Luckily, however it is at this stage that cyst formation takes place, and while the infection is maintained by the multiplication of these smaller amœbæ by simple division, as in the case of *E. coli*, certain individuals become encysted (figs. 34 to 37). The cysts escape from the intestine, and they have characters which enable them to be recognized fairly readily. They are spherical, but not so accurately spherical as the cysts of *E. coli*. They are smaller, having a diameter of 10 to 14 microns, and in accordance with the higher refractiveness of *E. histolytica* the cysts are more refractile, so that the contents are more difficult to see clearly. Nuclear multiplication leads to the production of two and then four nuclei, and only very rarely indeed eight. In the case of *E. coli* the cysts which escape have nearly all reached the eight-nuclear stage, whereas with the cysts of *E. histolytica* it is very usual to find the cysts voided in all stages of development. The fully-formed cyst of *E. histolytica* is then a spherical greenish refractile body 10 to 14 microns in diameter enclosing a single mass of cytoplasm containing four nuclei, which often lie in pairs at opposite poles of the cyst. Fairly frequently the cysts contain in addition to the nuclei one or two or more rods of a homogeneous highly refractile substance, which go by the name of the chromidial bodies, and, further, there may be a large vacuole in the cytoplasm. If only a single cyst is seen it may be difficult to diagnose with certainty, but a single fresh cover-glass preparation will often contain fifty or more, so that a definite opinion can easily be arrived at.

As in the case of *E. coli* the cysts are responsible for the spread of infection, so that an individual who has recovered from an attack of dysentery, but who is still harbouring the small amœbæ which are producing the cysts, is a carrier who is far more dangerous to the general public than an individual who is actually suffering from true amœbic dysentery. Apparently carriers may maintain their infections for long periods, during which time they are constantly passing cysts, sometimes in enormous numbers, and certainly spreading the infection in this manner. In a place like Gallipoli such infective cysts may be spread by any agent which will distribute them such as flies, water, wind, and possibly also dust. Fortunately a simple microscopic examination of the fæces, as explained above, will detect the condition, and a treatment by emetine injections will almost certainly get rid of it. On several occasions I have produced

typical and fatal amœbic dysentery in cats by administering fæces obtained from such carriers of infection, who have shown at the time no signs of amœbic dysentery.

Amœba limax (*Wahlkampfia limax*) (figs. 45 to 47).

In addition to the two common amœbæ described above there occurs sometimes in the large intestine of man a small amœba about ten microns in diameter. It resembles very closely free-living water amœbæ of the *Amœba limax* type, and it may be one of these present in the gut accidentally. Encysted forms of free-living amœbæ, which are almost as widely distributed as bacteria, are being constantly eaten with food, and it is supposed that under certain conditions not at present understood the amœbæ escape from the cysts in the large intestine and there multiply. As a rule, however, the cysts pass unchanged through the intestine. These amœbæ are recognized from the true entamœbæ by their small size and the character of the nucleus, which is a spherical body enclosing a very large central karyosome. Many attempts have been made to obtain cultures of *E. coli* and *E. histolytica* by inoculating various media with fæces containing them, and in certain cases cultures of amœbæ have been obtained, but the amœbæ in the cultures always have the characters of the small *limax* amœbæ and not those of the entamœbæ. It is probable that the cultures obtained, as I pointed out so long ago as 1907, and as has recently been conclusively demonstrated by Walker in the Philippines, have originated, not as supposed from the entamœbæ, but from the small *limax* amœbæ or their cysts, which have been undetected and accidentally present in the inoculated material.

In the large intestine these small *limax* amœbæ have no pathogenic significance and are recognized from the entamœbæ chiefly by their small size. They are, however, difficult to distinguish from certain intestinal flagellates which have become rounded and changed after having left the body for some hours, and furthermore it seems certain that flagellates belonging to the genera *Cercomonas*, *Bodo*, and *Prowazekia* have an amœboid stage which is indistinguishable from a typical *Amœba limax*.

Trichomonas intestinalis (figs. 20 to 26).

Of all the intestinal flagellates of man this is by far the commonest. It lives in the large intestine and cæcum, where it occurs sometimes in enormous numbers and varies very considerably in size. It has a pear-shaped body varying in length from

about 5 to 15 microns. At the blunt anterior end of the body is a spherical nucleus, just anterior to which is a chromatin granule from which arise three long free flagella which are directed forwards, and a fourth thicker flagellum which passes backwards in a slightly spiral manner attached to the border of an undulating membrane, beyond which it is continued at the posterior end of the animal as a free flagellum. Near the nucleus at the anterior end is a slight conical depression, the cytostome. Running along the base of the undulating membrane and arising from the granule from which the flagella spring is a stiff rod which may function as a stiffening rib for the membrane. Another structure, the axostyle, arises near the cytostome in the nuclear region. It is a clear refractile bar, which is continued through the body towards the posterior end, where it protrudes through the surface as a sharp point. The cytoplasm of the body is often vacuolated, and within the vacuoles are bacteria which appear to be ingested as food.

In the fresh condition the flagellates progress rapidly by vigorous lashing movements of the three anterior flagella, while the undulations of the membrane and the movements of the attached flagellum cause it to revolve on its longitudinal axis. The movements may be so rapid that the study of the organism is difficult. After some time the movements slow down, and then with the one-sixth or one-eighth inch objective the characters can be easily seen. Often when a preparation has been first made no flagellates are visible, but after a while they are seen to wriggle out of the thicker portions into the more open parts or streams of liquid, in which they swim about very rapidly. As they revolve the undulating membrane becomes visible when it is directed upwards. Even then some difficulty may be experienced in seeing clearly the anterior flagella, which are very fine structures. This difficulty can easily be remedied by examination with the dark-ground condenser, for then not only are the flagella clearly visible, but all the movements of the flagellate are beautifully seen. It is important to count the three anterior flagella, for it is only by this means that one can distinguish the *Trichomonas* from two other closely allied though much rarer forms—the *Tetratrichomonas*, which only differs in the possession of four anterior flagella, and the *Pentatrichomonas*, which has five.

Reproduction of the flagellate is by longitudinal division. The nucleus divides, the granule from which the flagella originate divides, a new undulating membrane is formed and a new stiffening rod grows out from the divided basal granule. The body of the flagellate with all the organs duplicated then splits, giving rise to

two daughter individuals. There is some doubt as to the reproduction of the axostyle, as some maintain that a new one is formed in each individual after absorption of the original one, while others think it divides longitudinally into two. As with the entamœbæ, the rate of successive divisions determines the size of the flagellate.

Trichomonas will survive for days after removal from the body, and even when none can be seen at first they will reappear after warming the fæces. They change in two ways; some of them degenerate by casting off and losing the various structures they possess, others without any such loss become spherical and motionless, and these, when warmed, will resume active life again. They are resistant bodies and will withstand the action of gastric juice for a considerable time, so that it appears probable that it is such contracted spherical forms which are responsible for the spread of infection, especially when it is remembered that definitely encysted forms are not known.

Those trichomonas which lose their various organs do so fairly regularly, and they assume a series of forms which may cause some confusion if not recognized. The first change is usually a breaking loose of the flagellum attached to the undulating membrane (fig. 23). It remains fixed at its point of origin, so that one has the appearance of an organism with one large thick flagellum and three finer flagella. The region of the membrane stills shows undulating movements. A further stage is the casting off of the large flagellum and the disappearance of the three finer anterior flagella. There is thus produced an irregular and constantly changing mass of cytoplasm, which still exhibits at one edge an undulating movement (fig. 24). These forms look like amœbæ with an undulating border, and it was undoubtedly these which led Castellani to describe the *Entamœba undulans* of the human intestine. Sometimes a peculiar movement is seen. At one end a long finger-like pseudopodium is suddenly thrown out. It moves backwards along one edge, getting shorter as it does so, till it completely disappears at the other end. Another one is at once formed, and the process is repeated at intervals of one or two seconds (fig. 25). Finally, it becomes a motionless mass of cytoplasm which is difficult to distinguish from a small *limax* amœba.

As regards the pathogenicity of *T. intestinalis* very little is known. The mere discovery of them in large numbers in diarrhœic conditions affords little evidence, as it is these very conditions which seem to favour their multiplication. It does not seem that in man infections are of long duration, for they have quickly

disappeared from the stools of individuals I have had under observation, and in this respect stand in marked contrast to infections with *Lamblia intestinalis*, *E. coli*, and *E. histolytica*, which appear to persist indefinitely. *Trichomonas* of the same or an allied species is often found in vaginal discharges. It seems most reasonable to assume this to be an invasion from the gut though the organism, which is larger than *T. intestinalis*, is generally given the distinctive name of *Trichomonas vaginalis*. One must always bear in mind the great variation in size of all the intestinal protozoa.

Trichomonas is very common in rats, mice, fowls, and other animals. Dr. A. C. Stevenson has shown me a section of the cæcum of a mouse in which there is a definite lesion of the mucous surface, which is being invaded by numerous *Trichomonas*. If such invasion can occur, probably through a surface broken by some other infection, or irritant such as sand, it is possible that the flagellates might aggravate the lesion or produce definite symptoms.

Escomel, working in South America, claims to have cultivated *Trichomonas* from cases of diarrhoea and to have produced infection with diarrhoea in dogs by injection of these cultures. Furthermore, he has claimed to have discovered the flagellate in the drinking water and that after clearing the reservoirs they were no longer found, while the dysentery ceased to be prevalent. This interesting work needs confirmation.

Lamblia intestinalis (figs. 1 to 8).

This flagellate is of fairly common occurrence and differs from other protozoa of the human intestine in that it lives in the upper part of the small intestine. In shape it resembles a pear split into two parts along the longitudinal axis. There is a flat surface on which there is a sucking disc with raised edge and a convex surface. The tapering extremity or tail can be turned over the convex back and it terminates in two flagella. There are three other pairs of flagella, the arrangements of which are best seen by referring to the plate. All four pairs of flagella originate in a paired rod-like organ occupying a central position in the flagellate. In stained specimens this paired rod-like structure can be resolved into a series of fibres and granules of a complicated nature, as shown in diagram (fig. 2). Two nuclei are present, one on each side of the rods, and these give the organism when viewed on the flat surface a curious face-like appearance. The flagellate swims rapidly and

on account of its shape moves about with a swaying motion in a manner reminding one of a flat-fish swimming in a tank of water. In the intestine it is able to rest on the surface of the epithelium with the flat, sucker-like disc applied to the cells and with its tail turned up over its back. Judging from the appearances I have seen in sections of the intestine of rabbits infected with *Lambli*a it would seem that the *Lambli*a invade the digestive glands of the mucous lining of the gut, where they can sometimes be seen sitting on the glandular epithelium in rows.

*Lambli*a *intestinalis* varies in length from about twelve to eighteen microns, and on account of its characteristic appearance there is little difficulty in recognizing it. It does not survive any length of time after escape from the body. At first very active, the movements gradually subside and consist only in lashings of the flagella. These gradually cease moving, but the two larger central flagella can often be observed gently undulating long after all other signs of life have disappeared.

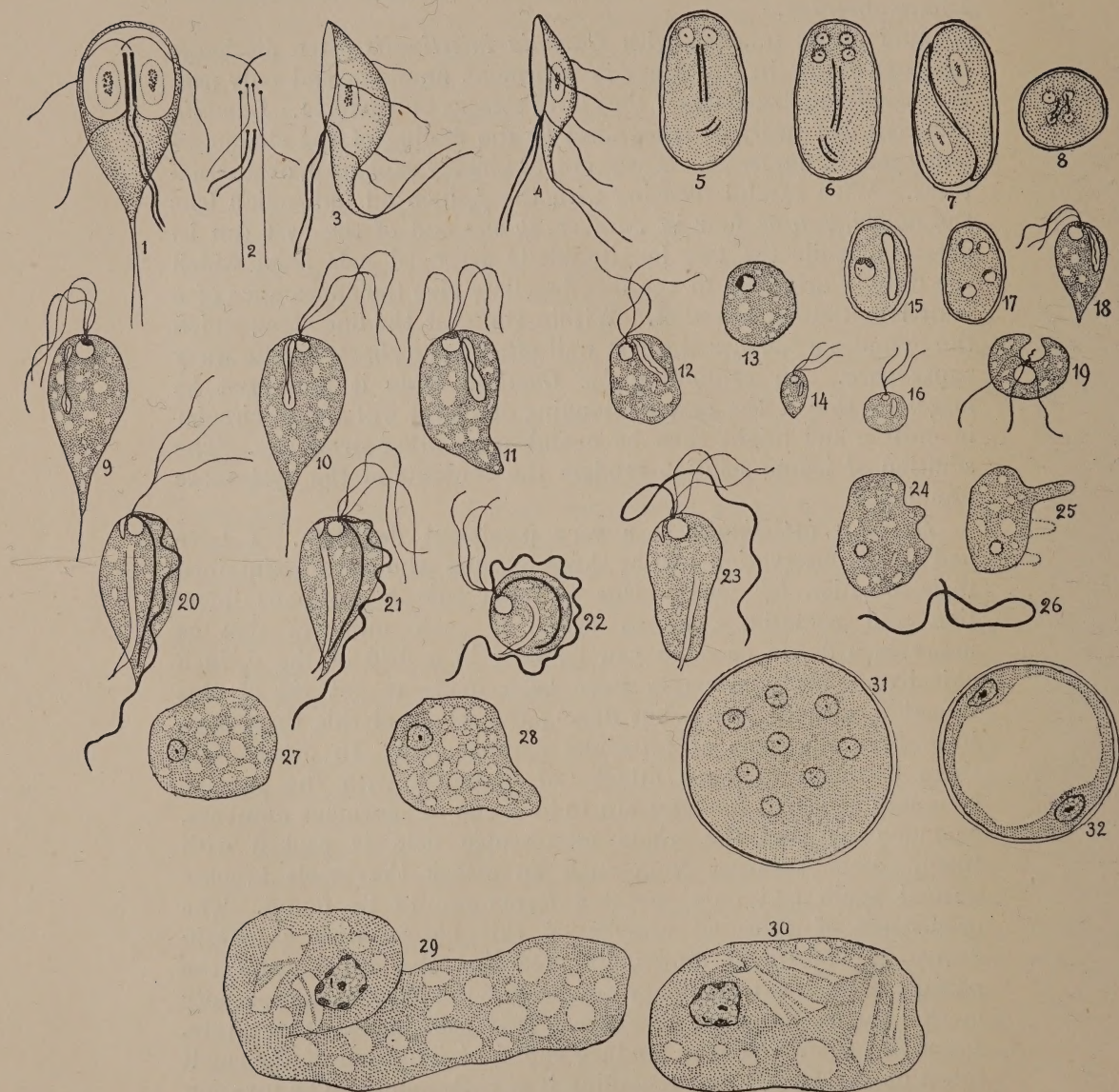
Reproduction of *Lambli*a appears to take place only in the encysted condition. I have never seen an unencysted dividing *Lambli*a, though I have observed these organisms constantly for many years. Multiplication takes place in a cyst, which is formed in the upper part of the small intestine. The cyst, which is formed round a single *Lambli*a, is an oval structure about fourteen microns in length. It is quite transparent like the cysts of the *Entamœbæ*, so that the *Lambli*a can be seen within it. Within the cyst certain changes of a very complicated nature take place. Firstly, the two nuclei migrate to one end of the cyst and there divide, so that a total of four are present (figs. 5 and 6). The complicated flagellar apparatus is duplicated and two nuclei migrate to the opposite end of the cyst. The contents of the cyst divide to form two *Lambli*a, which can be seen in the cyst with the oblique line of separation between them (fig. 7). Apparently, if reproduction has proceeded thus far while the cyst is still in the small intestine it ruptures and the two daughter *Lambli*a escape and help to maintain the infection of the small intestine. If, on the other hand, the cyst has passed into the large intestine before complete development has taken place it ceases to develop further and is passed out to the exterior, where it awaits ingestion by a new host. If it gains entrance to the intestine of such a host the development is completed, and two *Lambli*a escape and bring about infection of the small intestine. The cysts of the *Lambli*a thus serve the double purpose of reproduction within the host and transmission of infection from one host to

another. It is possible that two types of cyst exist, but the account I have given seems to be the simplest explanation of the appearances I have observed.

Individuals infected with *Lamblia intestinalis* often discharge encysted forms in the faeces in enormous numbers, and only pass the free-living flagellates if they have attacks of diarrhoea. *Lamblia* infection can easily be recognized by the finding of the characteristic cysts in the faeces. These are, as already explained, oval structures. With careful focusing with the $\frac{1}{12}$ -inch oil immersion lens the nuclei, two or four in number, at one end of the cyst can be detected, while the two longitudinally arranged rods from which the flagella originate in the free flagellate give the appearance of a faint longitudinal striation. Within the cyst the line along which the cytoplasm touches the cyst wall often has a characteristic wavy appearance. In examining for *Lamblia* cysts it must not be forgotten that if the cyst is standing on end it will appear circular in outline and might then be mistaken for other structures. The addition of iodine solution renders the contents of the cyst more prominent.

Lamblia intestinalis is a very persistent flagellate. I have had under observation two or three persons who have maintained their infection for years. One of these has an enormous infection and sometimes passes cysts in such numbers that as many as a dozen or more can be seen in a field of the $\frac{1}{12}$ -inch objective. Many attempts have been made to get rid of this infection without result, but during the whole of this time there have been no signs of intestinal derangement. In other cases there occur at intervals attacks of diarrhoea with the passage of mucus in which *Lamblia* are to be found in enormous numbers, so much so that the whole microscopic field is packed with them. After recovery from such an attack the stools become normal again and only encysted forms are to be found. The occurrence of repeated attacks of this nature with a certain degree of abdominal uneasiness preceding the attacks and the passage of such extraordinary numbers of the flagellates, especially in the mucus, leads me to suspect that sometimes, at any rate, *Lamblia intestinalis* may produce sufficient irritation of the small intestine to justify us in regarding it as pathogenic. The invasion of the glands of the small intestine as seen in the rabbit is suggestive of such a pathogenic rôle.

In the way of treatment injections of emetine apparently have no effect. A case recently came under the notice of Dr. G. C. Low



DESCRIPTION OF FIGURES.

All the figures have been drawn to one scale as shown, with the exception of figs. 54 and 55, which are only half the size they should be. An ordinary human red blood corpuscle on same scale is shown at figs. 48 and 56 for comparison.

Lambia intestinalis (figs. 1 to 8).

- (1) Surface view showing sucking disc, two nuclei, and eight flagella.
- (2) Origin of flagella as seen in stained preparations. They are represented as being more spread out than is actually the case.
- (3) Side view of thick form.
- (4) Side view of narrow form.
- (5) Encysted form with two nuclei.
- (6) Encysted form with four nuclei.
- (7) Encysted form containing two flagellates.
- (8) Appearance of cyst when viewed on end. The cysts are sometimes shorter in proportion to their breadth and much more definitely egg-shaped, with one end slightly narrower than the other, than represented in the plate.

Tetramitus mesnili (figs. 9 to 19).

- (9) Form with overlapping lips of cytostome.
- (10) Form showing flagellum in cytostome.
- (11) Form in which posterior filamentous extremity is retracted.
- (12) Still further retracted form.
- (13) Rounded form in which flagella are lost, so that the resemblance to a small amœba is marked.
- (14) Very small form of normal shape.
- (15) Encysted form with single nucleus and cytostome visible.
- (16) Very small round form.
- (17) Possibly encysted form with four nuclei.
- (18) Intermediate form of normal shape.
- (19) Appearance of flagellate when viewed on end, the cytostome with the incurved lips shown clearly, as also the flagellum within.

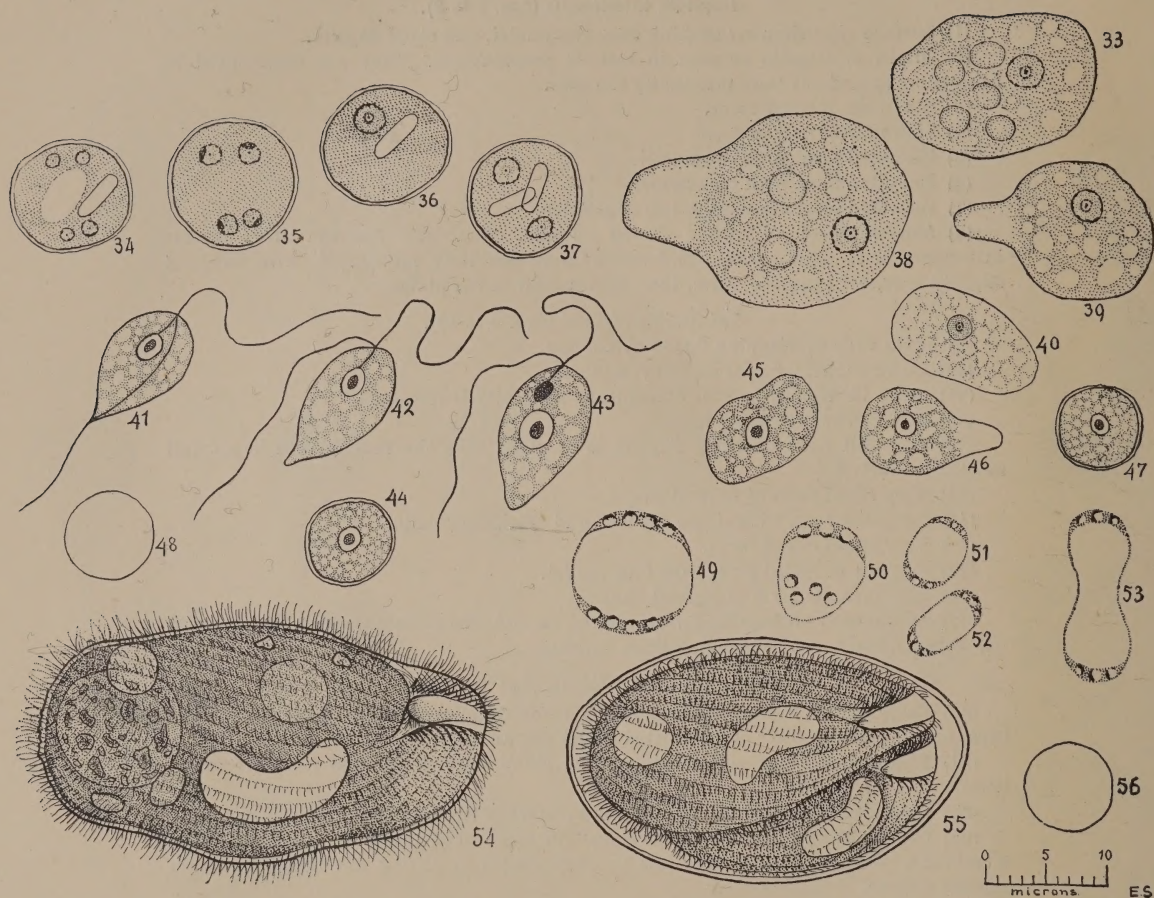
Trichomonas intestinalis (figs. 20 to 26).

- (20) Flagellate of normal structure; the three flagella appear to have a common base, possibly due to their being twisted round one another.
- (21) Flagellate of normal structure; the three flagella are free in their entire length.
- (22) Rounding off form with undulating membrane running round margin.
- (23) Degenerating form; the large flagellum has broken loose from the undulating membrane, so that the flagellate has the appearance of having one large and three smaller flagella.
- (24) Further degeneration; the flagella and axostyle are lost, so that the appearance is of an amœba with an undulating border.
- (25) Amœboid form throwing out the finger-like pseudopodium, which rapidly passes down side of body into dotted position, where it disappears.
- (26) Detached flagellum.

Entamœba coli (figs. 27 to 32).

- (27) Small entamœba of roughly spherical form and vacuolated cytostome.
- (28) Small entamœba forming pseudopodium with no distinction between ecto- and endo-plasm.
- (29) Large entamœba of irregular shape.
- (30) Large entamœba with slit-like rectangular vacuoles.
- (31) Encysted form as it appears in the fæces. This is the form most commonly observed and which is most useful for diagnostic purposes.
- (32) Encysted form of abnormal type with large central vacuole. In other cases there may be several vacuoles, and the vacuolation has the effect of retarding nuclear division, as such forms usually have only two or possibly four, nuclei.

In *E. coli* infections it is generally only the completely developed cyst with eight nuclei which is passed in fæces. The earlier stages of development with one, two, and four nuclei take place in the large intestines before the cysts escape.



Entamoeba histolytica (figs. 33 to 40).

- (33) Large tissue-invasive form (*tetragena* form) containing five red blood corpuscles.
 (38) Large tissue-invasive form with ectoplasmic pseudopodium and containing two red blood corpuscles.
 (39) Small form of intermediate size with ectoplasmic pseudopodium.
 (40) Small *minuta* form as seen in post-dysenteric conditions.
 (34) Encysted form with four nuclei, chromidial body, and vacuole.
 (35) Encysted form with four nuclei. It is distinguished by its smaller size from the four-nuclear stage of *E. coli*, which, however, is rarely passed in the faeces.
 (36) Encysted form with one nucleus and chromidial body.
 (37) Encysted form with two nuclei and two chromidial bodies.

The encysted forms begin to appear as the acute dysenteric symptoms subside, and are thus very characteristic of the infection in carrier cases. It is important to note that they are much smaller than the cysts of *E. coli*. In *E. histolytica* infections it is usual to find passed in the faeces cysts in all stages of development.

Cercomonas, Bodo, Prowazekia (figs. 41 to 44).

- (41) *Cercomonas*. The backwardly directed flagellum is adherent to the body. There is only a single nucleus.
 (42) *Bodo*. The two flagella are free and there is only a single nucleus.
 (43) *Prowazekia*. The two flagella are free and there are two nuclei.
 (44) Encysted form of any of above three flagellates.

Amœba limax (figs. 45 to 47).

- (45) Form without pseudopodium and characteristic *limax* nucleus.
 (46) Form with pseudopodium.
 (47) Encysted form.
 (48) Red blood corpuscle to show relative size of objects in plate.

Blastocystis hominis (figs. 49 to 53).

- (49) Large spherical form with several nuclei in semilunar protoplasm at opposite poles.
 (50) Somewhat triangular form with many nuclei.
 (51) Small oval form.
 (52) Small elongated form.
 (53) Elongated dividing form.

This organism is of a vegetable nature, but under certain conditions degenerating flagellates and small amœbæ or the encysted forms of these, by the development of a large central vacuole, will closely simulate the true *Blastocystis*.

Balantidium coli (figs. 54 to 55).

- (54) Free ciliate as it lives in lumen of gut and in tissues.
 (55) Encysted form containing two ciliates as passed in fæces.
 (56) Red blood corpuscle as in fig. 48. It is represented as a corpuscle on an ordinary blood film where it is somewhat flattened out and appears larger than it really is (compare corpuscles within amœbæ in figs. 33 and 38).

and myself. It was a man who was a carrier of *E. histolytica* and *Lamblia intestinalis*. He was passing large numbers of cysts of both kinds. He was treated by injections of emetine, which had the effect of completely ridding him of his entamœbic infection, but had no effect on the *Lamblia*. Subsequent treatment with large doses of β -naphthol reduced the infection, but failed to get rid of it; β -naphthol, however, appears sometimes to destroy a *Lamblia* infection of the intestine. Another case of *Lamblia* infection was treated by doses of emetine in capsules by the mouth, also without result. This case again failed to respond to treatment with β -naphthol. The somewhat drastic treatment adopted for the destruction of *Ankylostoma duodenale* in the duodenum has also failed to destroy completely the *Lamblia* in the small intestine. It is possible that the forms in the cavity of the gut come under the influence of the drug, while such as are lurking in the tubular glands escape entirely, and are responsible for the re-establishment of the infection after treatment had ceased.

Tetramitus mesnili (*Macrostoma mesnili*) (figs. 9 to 19).

This is a flagellate which was first found by me a few years ago in the intestine of a man who had come to London from the Bahamas. He had been admitted to the Albert Dock Hospital for some chest complaint, and the flagellate was only discovered in the course of routine examination.

In general shape, size, and the possession of three long fine anterior flagella it resembles *Trichomonas intestinalis*. It differs, however, in having no undulating membrane and no axostyle. It has, on the other hand, a large cytostome in the form of a longitudinal slit, which runs from the anterior end along half or two-thirds of the body length. The cytostome has a sharp margin which is sometimes produced into lips which appear to overlap one another (fig. 9). Within the cytostome is a longitudinal flagellum, which seems to be attached to the border of an undulating membrane (fig. 10). The cytostome flagellum and the three anterior flagella all arise from a granule which lies just anterior to a spherical nucleus near the anterior end of the cytostome. The posterior end of the animal is pointed and may sometimes be drawn out to a length equal to, or greater than, that of the body itself. The cytoplasm is generally very vacuolated and contains bacteria, which seem to be the staple article of diet. The size of the flagellate varies considerably, there being minute forms three or four microns in length, and every intermediate gradation up to individuals with a length of about fifteen microns.

There seems to be some doubt as to the method of reproduction of *Tetramitus mesnili*. I have never seen an undoubted dividing form in a stained film, but some such method of multiplication must occur, and the presence in fresh preparations of specimens with two tail-like prolongations may be an indication of multiplication by simple longitudinal division such as occurs in the case of *Trichomonas*.

Encysted forms of *Tetramitus* are produced in the shape of oval transparent cysts seven to eight microns in length (fig. 15). Within the cysts the characteristic features of the *Tetramitus* can be seen. The future of these cysts is probably escape from the body and transmission of infection to other individuals, as with so many of the intestinal protozoa. In some preparations I have seen cysts in which a single nucleus has divided twice to produce four nuclei, so that it seems probable that after encystment multiplication takes place within the cyst (fig. 17). The

development of the cysts of *Tetramitus* and its method of multiplication within the gut require further study.

Tetramitus mesnili is readily seen with the one-sixth inch objective. It can most easily be confused with *Trichomonas*, though the undulating membrane of the latter and the large cytostome of the former should prevent such an error. The counting of the three anterior flagella offers the greatest difficulty, and, as with *Trichomonas*, they are most clearly seen with dark-ground illumination. This method of observation also affords the clearest view of the movements of the flagellum within the cytostome—a flagellum which is so difficult to detect with ordinary transmitted light that some have even denied its existence.

Like other flagellates of the intestine the activities of *Tetramitus mesnili* are greatest in fæces which have been freshly passed. Soon the movements become less active and finally cease completely. *Tetramitus* survives in fæces only a few hours, and in this respect is quite unlike *Trichomonas*, which may survive for days. The normal pear shape of the organisms is quickly lost and all kinds of distorted forms soon appear (figs. 11 and 12). Some lose their flagella and contract till they are mere spherical masses of cytoplasm, which are then practically indistinguishable from the spherical forms of *Trichomonas* or of small amœbæ of the *limax* type. It is possible that this assumption of the spherical form has to do with an attempt at encystment, but the actual encystment of such forms has not been observed.

Tetramitus mesnili was first discovered by me in a man with no intestinal symptoms. Since then it has been found to have a very wide distribution in tropical and sub-tropical countries, and certain small epidemics of diarrhœa have been attributed to this flagellate. In all these cases in which *Tetramitus* and, indeed, all intestinal parasites are present, the greatest care should be taken to exclude all bacterial infections such as typhoid, paratyphoid, dysentery, etc., for the very gut conditions produced by these bacteria may be the cause of inducing an active multiplication of perfectly harmless flagellates.

Cercomonas, Bodo, Prowazekia (figs. 41 to 44).

There are three other flagellates which should be mentioned as they occur, though in my experience very rarely, in the human intestine. These are species of *Cercomonas*, *Bodo* and *Prowazekia*. It is doubtful if they are true parasites like the flagellates described above, for they correspond very closely and are possibly identical

with free-living forms which are often to be found in wet, decomposing material. They will often appear in fæces which have been kept a few days, though they could not be detected in the freshly passed fæces. They are all three slightly elongated flagellates up to 15 microns in length, with a blunt anterior end and a tapering posterior end. They each possess two flagella, one of which is directed forwards in movement, while the other trails backwards. Both flagella take origin at the blunt anterior end of the body. In both *Bodo* and *Prowazekia* (figs. 42 and 43) the two flagella are free, while in *Cercomonas* (fig. 41) the trailing or backwardly directed flagellum is attached to the surface of the body as far as the posterior end of the flagellate, when it is continued as a free flagellum. *Cercomonas* and *Bodo* have each a single nucleus, consisting of a nuclear membrane and large central karyosome. The flagella appear to take origin from the nuclear membrane. In *Prowazekia* (fig. 43), on the other hand, there are two nuclei, from one of which the flagella arise. The general structure of these flagellates will be seen on the diagram.

The three flagellates just described have probably no pathogenic significance whatsoever, and are to be regarded as free-living forms which have taken up a temporary abode in the gut. They are very readily cultivated on agar or liquid media in which bacteria are growing and there may become encysted in small spherical cysts 6 to 8 microns in diameter (fig. 44). They probably all have an amœboid stage indistinguishable from *Amœba limax*. The cysts are probably responsible for the occasional appearance of the flagellates in the intestine of man, for cysts of these forms constantly pass through the intestine, and it is the flagellates which escape from these cysts in fæces that are kept for a few days which give rise to the cultures of flagellates. Like the small *limax* amœbæ described above, they may occasionally escape from their cysts while they are still in the large intestine, and thus give rise by rapid multiplication to a temporary infection of the gut.

That these forms are of rare occurrence is shown by the fact that only in one case in ten years' experience of examinations for intestinal protozoa have I come across undoubted *Cercomonas*. I must make one remark about the name *Cercomonas*, which is used very loosely in medical literature. It has become a habit with some to call any actively moving flagellate seen in fæces a *Cercomonas*, quite regardless of the fact that the true *Cercomonas* is a flagellate of very definite structure. In the diagnosis of intestinal flagellates it is absolutely essential to see clearly the structure and

number of flagella, for without these data it is impossible to arrive at a clear idea of which flagellate one is dealing with. To one experienced in the examination of such organs the ordinary transmitted light will be sufficient, but, as already mentioned above, the dark-ground illumination is a great help. Staining may be of some assistance, and I will give at the end of this paper some general directions for carrying out this process for intestinal protozoa.

Balantidium coli (figs. 54 and 55).

This protozoon is a member of the group Ciliata, and is the only common ciliate of the human intestine. It appears to be a normal inhabitant of the intestine of pigs, and those who have to deal with these animals are most liable to infection. It inhabits the large intestine of man, and has the power of boring its way into the mucous coat and producing extensive ulceration, very much as *E. histolytica* does. It is sometimes found in the mesenteric glands draining the ulcerated areas. The condition produced by this ciliate is known as balantidial dysentery.

Balantidium coli is a large organism varying considerably in size, according to Brumpt. Its length may be anything from 30 to 200 microns and its breadth 20 to 70. On an average it is about 50 to 100 microns in length. It is thus a much larger organism than any of the other intestinal protozoa. It has an ovoid body which is slightly narrower at the anterior than at the posterior end. There is a cytostome opening at the anterior end; the whole body is covered with longitudinal rows of cilia which are longest around the cytostome. There is a large nucleus which is usually elongated and slightly curved (horseshoe-shaped), the macronucleus and a smaller nucleus, the micronucleus, which is generally hidden in a depression in the macronucleus. There are present in the cytoplasm two contractile vacuoles which pulsate at intervals, while there occur a varying number of food vacuoles of different sizes. Food is taken in through the cytostome and passes into vacuoles in the cytoplasm, where digestion takes place. Undigested particles of food are thrown out from the posterior end of the animal at the anus, which is only visible at the moment of extrusion of any particle.

These large ciliates swim about amongst the intestinal contents by means of their cilia, while the anterior narrower end exhibits a certain amount of change of shape as if the creatures were feeling their way through the débris. Multiplication both in the gut cavity and in the invaded tissues takes place by transverse

division, the nucleus first dividing into two parts. When multiplication is proceeding rapidly there may result small individuals not more than 30 microns in length, as Brumpt has shown in the case of experimental infection in monkeys.

As with other intestinal protozoa, cysts are produced, which are responsible for the infection of new individuals. According to Brumpt, these cysts may be formed round single *Balantidia*, or two may enter a single cyst. In the latter case a conjugation takes place by fusion of the two ciliates. The cysts of *Balantidium* are roughly spherical or oval structures about fifty to sixty microns in diameter; they can be recognized by the ciliated organism within.

Balantidium coli is such a large and characteristic organism that there can be no difficulty in its recognition. It might possibly be confused with ciliated embryos escaping from worms' eggs if careful observation is not made.

Other ciliates have been met with in the intestine of man, but many of these are only accidental infections with ciliates which usually live in water and are in no way parasitic. A small ciliate measuring 20 to 30 microns in length was described by Schaudinn from the human intestine under the name of *Balantidium minutum*. It has the same general structure as *Balantidium coli*. Another described by the same author is *Nyctotherus faba*, a ciliate with a lateral instead of a terminal cytostome. Both of these ciliates are of rare occurrence and are probably of no pathological significance.

Blastocystis hominis (figs. 49 to 53).

The description given above includes all the protozoa which are likely to be encountered in the intestines of people returning to this country from tropical or subtropical countries, but it must be remembered that faeces contain vegetable cells, yeasts, and other bodies which must not be confused with encysted protozoa. One such organism of a vegetable nature is known as *Blastocystis hominis*. It is a more or less spherical structure which varies in diameter between 5 and 15 microns, and may fairly readily be mistaken by the inexperienced for encysted *E. coli* or *E. histolytica*. The Blastocystis is a very much more flimsy structure with more delicate capsule than the cysts of entamoebæ. The great part of the cyst content is a large vacuole which reduces the cytoplasm to a narrow rim at one or often two poles of the cyst. In this narrow, greenish rim of cytoplasm are to be seen a varying number of

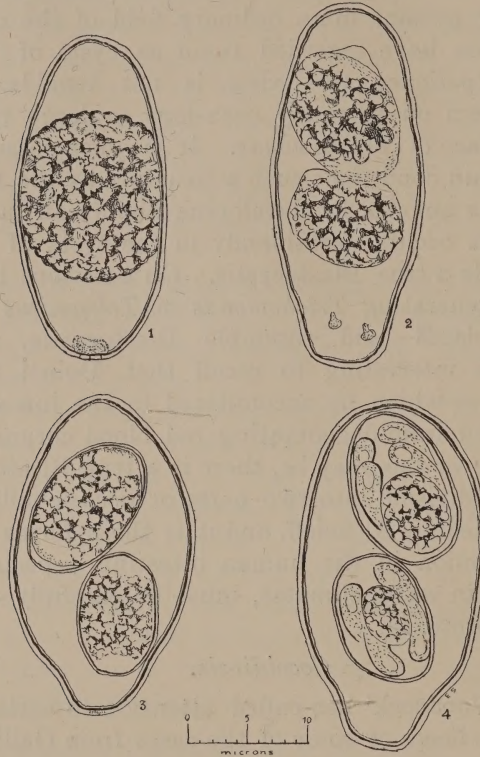
refractile, greenish spots which are really nuclei. These spherical Blastocystis may become elongated and divide transversely into two parts. Alexeieff regards them as vegetable organisms allied to the yeasts. Sometimes they occur in enormous numbers in the stool—hundreds being present in an ordinary field of the one-sixth inch objective. Some have regarded them as cysts of *Trichomonas*, but in my experience this view is not tenable, for I have encountered them on so many occasions in large number when there was no trace of *Trichomonas*. It is quite possible, however, that under certain conditions such a structure as the spherical cyst of a small *limax* amoeba, by developing abnormally a large central vacuole, such as occurs occasionally in the cysts of *E. coli*, may come to resemble a true Blastocystis. On occasions it has seemed to me that degenerating *Trichomonas* or *Tetramitus* may become centrally vacuolated and resemble Blastocystis, and in this connexion it is interesting to recall that Dobell thought that similar structures which he encountered in the intestines of frogs had been derived from degenerating red blood corpuscles of these animals. However this may be, there is a true Blastocystis which can multiply by division into two parts or occasionally by multiple division as claimed by Alexeieff, and it is these forms which, being exceedingly common in the human intestine, especially of those who have lived in warm climates, must be carefully distinguished from encysted protozoa.

Coccidiosis.

Dr. H. M. Woodcock¹ has called attention to certain bodies he had found in the fæces of some of the cases from Gallipoli. These were elongated cysts containing one or two masses of protoplasm. He considered them to be oöcysts of a coccidium and thought they were related to the isospora. He was unable to obtain any further development of the bodies, so that their coccidial nature was unproved. Since Woodcock's paper appeared Dr. G. C. Low has come across one case and I have seen three others at the London Hospital. In the fæces of one of these kept at the laboratory temperature the cysts have completed their development in three to four days. The single mass of protoplasm contained by the oöcysts divides into two sporoblasts, which in their turn become enclosed in oval sporocysts. Within the sporocyst each sporoblast now divides into four sporozoites and a large residual body, so

¹ *British Medical Journal*, November 13.

that the fully developed oöcyst contains two sporocysts, each of which contains a residual mass of cytoplasm and four sporozoites. The oöcyst when passed in the fæces contains the single mass



Human Coccidiosis.

Extracorporeal development of the human coccidium. The intracorporeal development of the coccidium takes place in the epithelial cells of the gut, there being an asexual cycle followed by the production of male and female sexual forms. Fertilization of the female gamete takes place in the gut and the zygote so formed becomes closed in an oöcyst, which is passed in the fæces and completes its development on the ground or in water. The first stage in the development is the retraction of the protoplasm so that it does not completely fill the cyst (fig. 1). Next, two sporoblasts are produced (fig. 2) by division of the single mass, and then each of these becomes enclosed in a sporocyst (fig. 3). The final stage is the production in each sporocyst of four sporozoites and a residual mass of cytoplasm (fig. 4). When this stage is reached, and not till then, the oöcyst is infective, and if swallowed the sporozoites escape, enter the epithelial cells of the gut, and recommence the asexual cycle. The oöcysts of coccidia are generally very resistant structures which can withstand a great amount of drying.

of protoplasm. The wall of the oöcyst shows a marked double contour and within it what appears to be a fine lining membrane. One end of the oöcyst often shows a narrowing just before the

end is reached, and at this end there is some indication of an opening, a micropyle through which in all probability the male gamete enters to effect fertilization. The micropyle is sometimes seen covered on the inner surface by a plug. The development, which I have followed, proves that Woodcock's conjecture was correct, and that these structures are really coccidia and belong to the genus *Isospora*, one member of which is a parasite of the intestines of cats and dogs, and another of the kidneys of frogs. As the coccidium develops in the intestinal epithelium it, of course, brings about destruction of the epithelial cells themselves, and so must be regarded of some pathogenic importance, although the symptoms of human intestinal coccidiosis have not been definitely determined. In animals such infections are often the cause of serious enteritis, which may have a fatal termination.

Methods of Examination.

The intestinal protozoa are best examined fresh and living and so soon after leaving the body as possible. The encysted forms protected by the cyst do not change and can be satisfactorily studied days after they have been passed, but the unencysted motile forms quickly change and degenerate, so that after some hours it may be a matter of great difficulty to identify them. As I have already remarked, it is better to examine several thin preparations of diluted fæces than one thick one, and if the fæces are perfectly fresh there is no necessity to use a warm stage, which may even be a disadvantage in causing the flagellates to move too rapidly for satisfactory observation. If the fæces are old a warm stage may revive amœbæ or flagellates which have ceased to move and thus help diagnosis.

Practically all details of structure which I have described above can be seen in the living forms, and there is no need to prepare stained preparations for this purpose. Great assistance in the diagnosis of flagellates is derived from the use of the dark-ground illumination.

Another point worthy of note is that the protozoa degenerate much more rapidly if the fæces be kept warm in an incubator. It is probably due to more rapid action of the bacteria at higher temperatures. Accordingly, if it is impossible to examine a specimen at once it is better to preserve it in the cold, and only warm it on the stage at the time of observation.

Often in dealing with the encysted forms of protozoa in fæces

it will be found that the nuclei within the cysts are difficult to distinguish. This is especially the case with the cysts of *E. histolytica* or *Lamblia*. The following method will be found useful in bringing the nuclei into greater prominence. A small drop of iodine solution (Weigert's solution = iodine 1, potassium iodide 2, distilled water 100) is placed on a slide and some of the fæces to be examined is rubbed in it by means of a match or platinum loop to give a yellow emulsion. A cover-glass is placed on it and the preparation examined at once. The cysts are stained a light-brown colour, and the nuclei, which may have been invisible before, now stand out clearly and can readily be counted.

If it should be desired to make permanent stained preparations the most satisfactory results are obtained by using some method of wet fixation. Ordinary dried smears stained by Giemsa stain will sometimes give fairly good pictures of the flagella of *Lamblia* or other flagellates, but, as a rule, so much distortion takes place that it is almost impossible to recognize the organisms.

The following method has given me very good results. A thin smear of the fæces, diluted if necessary, is made on a cover-glass and this is dropped without drying, film side downwards, on to a fixing fluid. A very good one consists of a mixture of two parts of saturated watery sublimate and one part of alcohol. The cover glasses float on the surface of the fixative and are allowed to remain there for twenty to thirty minutes. They are then carefully removed and placed in a Petri dish of thirty per cent spirit (this time film side up) in order to remove the sublimate. Great care must be taken to prevent them scraping against one another. After a few minutes' washing in this manner in several changes of the alcohol they are placed in distilled water and are ready for staining, and are to be treated as if they were sections. The best results are obtained by staining with iron hæmatoxylin. The films are left to soak for some hours (during the day or over night) in a four per cent solution of iron alum. They are then washed for a second or two in distilled water and placed in Heidenhain's hæmatoxylin,¹ in which stain they become quite black. They are left there for several hours as before. The black films are then washed in distilled water and placed in a one per cent iron alum

¹ To prepare the stain dissolve 1 gramme of hæmatoxylin crystals in 10 cubic centimetres of absolute alcohol by warming; add 90 cubic centimetres distilled water and allow to mature for a week to ten days, and then add a further 100 cubic centimetres distilled water.

solution, which commences to dissolve the black stain. The differentiation must not be carried too far, and in order to control it the films must be examined every few minutes in distilled water with a water immersion lens or with the ordinary $\frac{1}{6}$ -inch objective. The success of the method depends on the right degree of differentiation, and in examination of the films it is essential to see the actual objects which are being stained, for objects vary in the amount of extraction required. The nuclei should show clearly as black rings. Experience alone can teach the right degree of extraction of the stain. As a rule, a flagellate film should not take longer than five to ten minutes for differentiation in one per cent iron alum solution. Objects like encysted forms of course take longer. When differentiation is complete the films are washed in distilled water and taken up through strengths of alcohol to absolute alcohol. They are then cleared in xylol and mounted in balsam. By this method of fixation and staining permanent preparations are obtained while the actual shape and structure of the organism are preserved and often every detail can be readily seen.

It is essential to have constantly at hand an eye-piece fitted with a micrometer scale, the size of the divisions of which is known in microns for each power of the microscope and for a definite tube length. If any object, as for instance a cyst of *E. coli*, is found, its size can at once be determined by inserting the micrometer eye-piece. The actual size of the divisions of the eye-piece scale is discovered by examining with this eye-piece the micrometer slide, on which a scale in tenths and hundredths of a millimetre is marked. It will be found that each division of the eye-piece scale with the $\frac{1}{12}$ -inch objective represents, say, 1.7 microns, and this is a constant factor which can be used at a moment's notice. With lower powers the value of each division will be correspondingly greater. It is much better to have a special eye-piece for this purpose so as to obviate the necessity of inserting the scale when required.

In the above description I have given an account of the common protozoa of the human intestine as I have seen them in the course of many years' observation, and it is hoped the description will be of some assistance not only to those who deal with the examination and treatment of cases in Egypt, Mudros, the Peninsula, and Mesopotamia, but also to others having under their care men in this country who have returned from localities where these infections are likely to be contracted.

I have recently seen a good many instances of protozoal infections of the gut in cases returning from Gallipoli in hospitals both in London and the provinces. *E. coli* is very common in these men, and also the flagellates *Trichomonas*, *Tetramitus* and *Lamblia*. No case of *Balantidium* infection has come under my notice, but one case has been recorded recently in Egypt. On the other hand, the vegetable organism *Blastocystis* occurs in large numbers in these cases, while occasionally I have seen a small amoeba of the *limax* type. I have seen only four cases of *E. histolytica* infection, and this is all the more gratifying, for I hear from Lieutenant-Colonel A. Balfour that such infections are far from uncommon out there, but that they are in most cases immediately cut short by suitable treatment with emetine. This speaks well for the wonderful action of this drug in killing off the pathogenic entamoeba and so preventing that formerly too common sequel of the disease, amoebic abscess of the liver.¹

APPENDIX.

In addition to the organisms found so frequently in the human intestine there has appeared in the faeces of some of the cases from Gallipoli a spherical body which is usually smaller but may be equal in size to the cysts of *Entamoeba histolytica*. It is probably of a vegetable nature and in faeces kept for some time there is an indication that it elongates and may reproduce by simple division or even grow into filaments or hyphae. It may thus be the spore of a fungus, but its importance is that it can be mistaken, especially when it is large, for the cysts of the pathogenic entamoeba. It is shown in the photomicrographs 7 and 8 of the plate. Each cyst has a single nucleus which is much smaller than the nucleus of the single nuclear stage of the *E. histolytica* cyst. In addition to the nucleus which appears in the fresh condition as a small granular ring there are one or more refractile bodies of varying size which again bear some resemblance to the chromidial bodies seen in cysts of *E. histolytica*. These bodies are very strongly iodophilic and in preparations of faeces made with Weigert's iodine solution they become a dark brown, almost mahogany colour, as shown in the

¹ Since writing the above I have extended my observations on men returned from Gallipoli and I have found that approximately ten per cent are harbouring *E. histolytica* mostly as carrier cases.

photomicrographs 7 and 8. The iodophilic body or bodies are often much larger and more irregularly shaped than shown in the plate.

The photomicrographs shown in the two plates have been made by Dr. A. C. Stevenson at the Wellcome Bureau of Scientific Research and they show more clearly and naturally than any drawing is able to do the appearance of some of the protozoal organisms of the human intestine. The photographs in Plate II show the invasion of the tissues by *E. histolytica* and *Balantidium coli* and it will be seen how these organisms spread into the submucous coat of the bowel. The photographs in Plate I are very excellent reproductions of the actual appearance of many of the cysts as they occur in fæces either mixed with iodine solution or with normal saline and they should be of great assistance to those who have to identify these bodies in fæces.

There still remains to be recorded another coccidium which I have found in one case from Gallipoli. A description of this form will appear shortly in the *Lancet*. It is quite distinct from the *Isospora* described above, and is a species of *Eimeria* (Coccidium), for the spherical oöcyst, which measures 20 microns in diameter, contains when mature four sporocysts, each of which encloses two sporozoites and a residual mass of cytoplasm. This coccidium resembles most closely *Eimeria falciforme*, a parasite of the intestine of the mouse.



PLATE I.

Various Cysts from Human Fæces.

Nos. 1 and 2 and 4 to 9 are from preparations of fæces rubbed up in Weigert's iodine solution. Nos. 3 and 10 to 13 are from fæces in normal saline solution. The magnification is about 1,000 diameters.

- (1) Cyst of *Entamæba coli*, showing five nuclei clearly and two less definitely.
- (2) Cyst of *E. coli*, showing five or six nuclei.
- (3) *Minuta* form of *E. histolytica* with ectoplasm and endoplasm.
- (4) Cyst of *E. histolytica* with one nucleus.
- (5) and (6) Cysts of *E. histolytica* with four nuclei.
- (7) and (8) Cysts probably of vegetable nature, each with single small nucleus and dark iodophilic body.
- (9) Cyst of *Lamblia intestinalis*.
- (10) to (13) Human coccidium, showing extracorporeal development into two sporocysts, each with four sporozoites.

PLATE I.

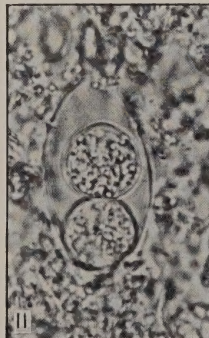
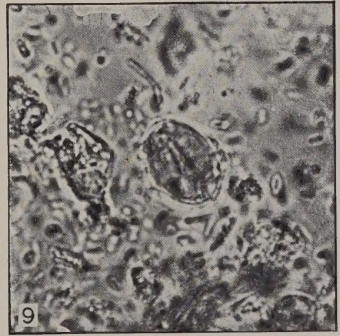
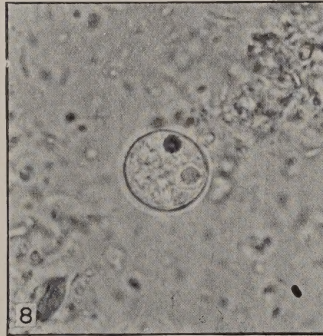
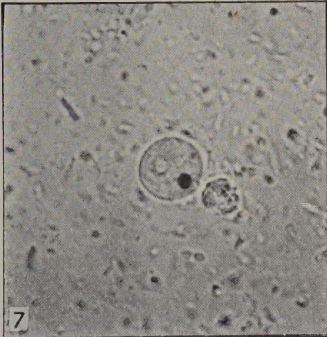
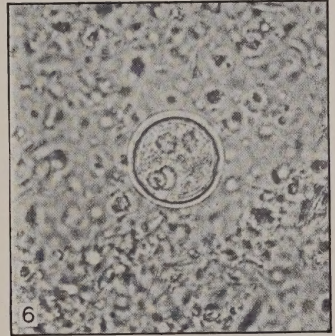
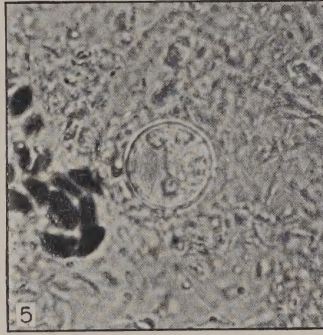
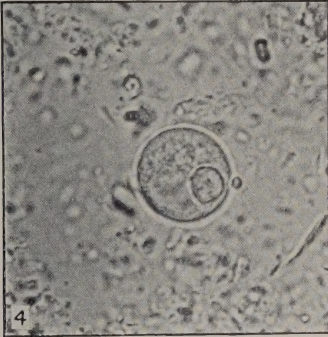
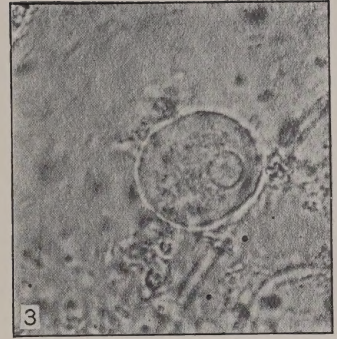
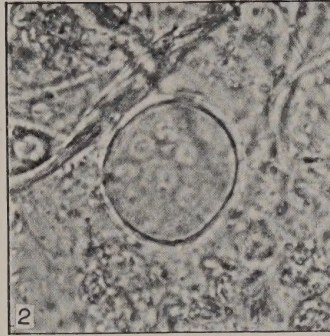


PLATE II.

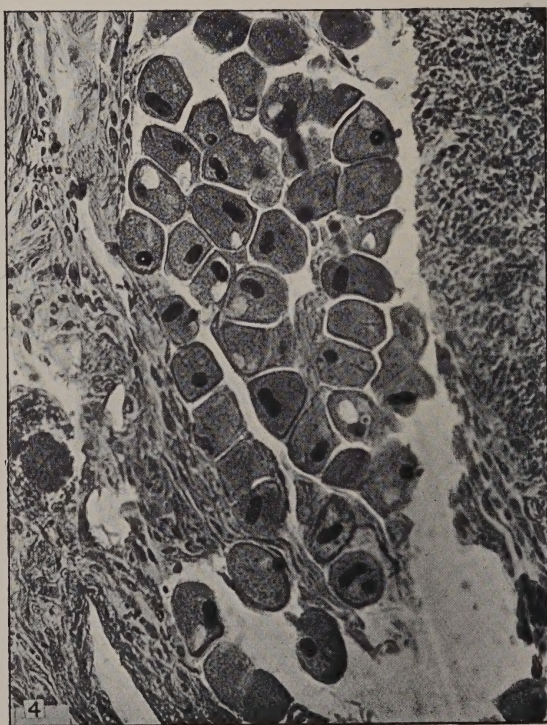
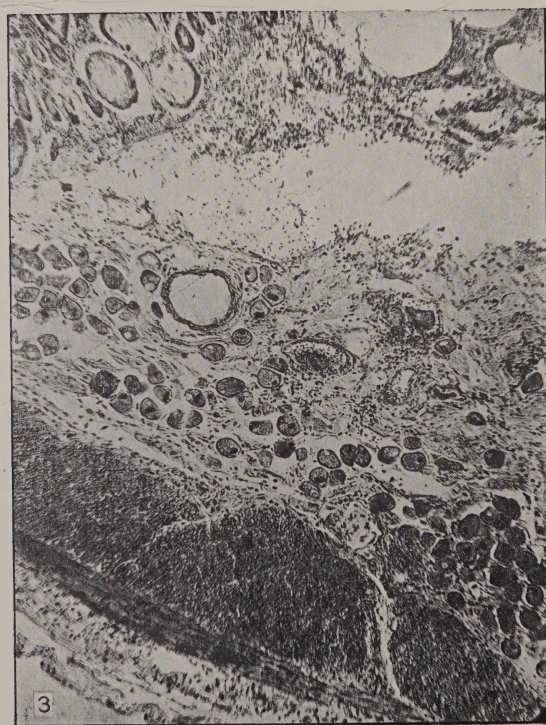
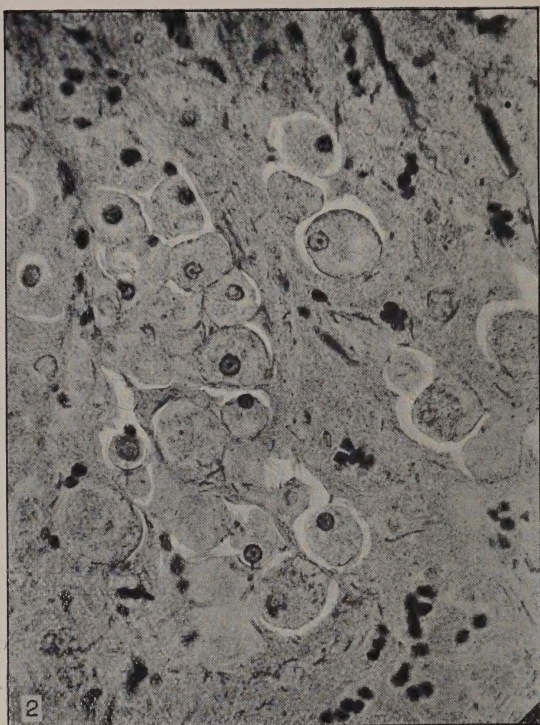
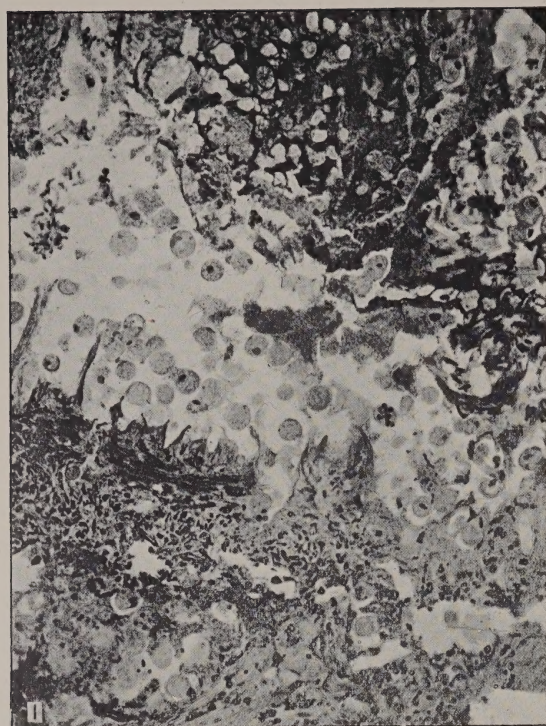


PLATE II.

(1) Section of gut in experimental infection of cat by *Entamoeba histolytica*, showing destruction of muscularis mucosæ and passage of entamœbæ from mucous to submucous coat. $\times$ *circa* 120.

(2) Section of very early ulcer in gut of cat showing entamœbæ in necrosed mucosa. $\times$ *circa* 500.

(3) Spreading edge of ulcer in balantidial dysentery of man. The Balantidia lie in the deep layers of the submucosa in the comparatively healthy tissue around the ulcer. $\times$ *circa* 55.

(4) Mass of Balantidia from similar section of human intestine. $\times$ *circa* 120.

